New benzo[*b*]pyrano[3,2-*h*]acridin-7-one compounds

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Title of the invention :

The present invention relates to new benzo[*b*]pyrano[3,2-*h*]acridin-7-one compounds.

Field of the invention :

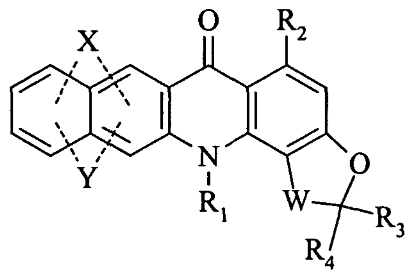
The compounds of the invention are derivatives of acronycine, an alkaloid which has anti-tumour properties that have been demonstrated in experimental models (*J. Pharm.. Sci.*, 1966, 55 (8), 758-768). However, despite having quite a broad spectrum of activity, acronycine is of low potency and moderate activity. The solubility of the compound is, moreover, low, which limits its bioavailability, as well as its use in pharmaceutical compositions for administration by the intravenous route.

Various modifications have been made to the molecule, for example those described in *J. Med. Chem.*, 1996, 39, 4762-4766 or EP 1 042 326, allowing a significant improvement in the potency, anti-tumour efficacy and solubility of the products. Nevertheless, anti-cancer therapeutic requirements call for the constant development of new anti-tumour agents with the aim of obtaining medicaments that are simultaneously more active and better tolerated. More specifically, solid tumours constitute a major problem for anti-cancer chemotherapy because of their intrinsic and/or acquired resistance to existing compounds. Moreover, certain compounds which are shown to be highly active with respect to certain cell lines are found to be inactive with respect to other cell lines or indeed toxic. It is therefore of prime importance to have access to the widest range of compounds exhibiting powerful cytotoxic activity in order to have available the most effective treatments for the totality of tumour disorders, together with limited secondary effects and the longest desirable action over time.

Besides the fact that the compounds of the invention are new, they have surprising *in vitro* and *in vivo* activity which is greater than that observed hitherto. The compounds discovered by the Applicant accordingly have anti-tumour properties that make them especially useful in the treatment of cancers and, especially, of solid tumours including lung and ovarian carcinoma.

Detailed description of the invention :

More specifically, the present invention relates to compounds of formula (I) :



(I),

wherein :

- **X** and **Y**, which may be the same or different, represent, independently of one another, a group selected from :
  - hydrogen and halogen atoms,
  - mercapto, cyano, nitro, linear or branched (C_1-C_6)alkyl, linear or branched (C_1-C_6)-trihaloalkyl and linear or branched trihalo-(C_1-C_6)alkyl-carbonylamino groups,
  - groups of formulae -ORa, -NRaRb, -NRa-C(O)-T_1, -O-C(O)-T_1, -O-T_2-NRaRb, -O-T_2-ORa, -NRa-T_2-NRaRb, -NRa-T_2-ORa and -NRa-T_2-CO_2Ra wherein :

  * **Ra** represents a group selected from a hydrogen atom and a linear or branched (C_1-C_6)alkyl group, an aryl group and an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched,

  * **Rb** represents a group selected from a hydrogen atom and a linear or branched (C_1-C_6)alkyl group, an aryl group and an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched,

  or

  Ra+Rb, together with the nitrogen atom carrying them, form a monocyclic 5- or 6-membered heterocycle optionally containing in the cyclic system a second hetero atom selected from oxygen and nitrogen,

  * **T_1** represents a group selected from linear or branched (C_1-C_6)alkyl, linear or branched (C_2-C_6)alkenyl, aryl, aryl-(C_1-C_6)alkyl (wherein the alkyl moiety is linear or branched), and a linear or branched (C_1-C_6)alkylene chain substituted by a group selected from -ORa and -NRaRb wherein Ra and Rb are as defined hereinbefore,

  * **T_2** represents a linear or branched (C_1-C_6)alkylene chain,

  - or X and Y, when they are in adjacent positions, together form a methylenedioxy group or an ethylenedioxy group,

it being understood that the substituents X and Y may be present on either of the two adjacent benzene rings,

- **R_1** represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,

- **R_2** represents a group selected from a hydrogen atom and linear or branched (C_1-C_6)alkyl, -ORa, -NRaRb, -NRa-C(O)-T_1, -O-C(O)-T_1, -O-T_2-NRaRb, -O-T_2-ORa, -NRa-T_2-NRaRb, -NRa-T_2-ORa and -NRa-T_2-CO_2Ra groups, wherein Ra, Rb, T_1 and T_2 are as defined hereinbefore,

- **R_3, R_4**, which may be the same or different, represent, independently of one another, a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,

- **W** represents a group of formula -CH(R_5)-CH(R_6)-, -CH=C(R_7)-, -C(R_7)=CH- or -C(O)-CH(R_8)- wherein :
  - **R_5 and/or R_6**, represent(s) a group selected from -W_1-C(W_2)-W_3-T_1, -W_4-C(W_2)-T'_1, -W_1-S(O)$_n$-W_3-T_1 and –W_1-S(O)$_n$-T_1 wherein :
    - W_1 represents an oxygen or sulphur atom or a nitrogen atom substituted by a hydrogen atom or by a linear or branched (C_1-C_6)alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched,
    - W_2 represents an oxygen atom or a sulphur atom,
    - W_3 represents an oxygen or sulphur atom or a nitrogen atom substituted by a hydrogen atom or by a linear or branched C_1-C_6) alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched, a bond when T_1 represents a linear or branched (C_2-C_6) alkenyl group,
    - W_4 represents a sulphur atom or a nitrogen atom substituted by a hydrogen atom or by a linear or branched (C_1-C_6)alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched,
    - T_1 is as defined hereinbefore,
    - T'_1 represents a group selected from linear or branched (C_2-C_6)alkenyl, aryl, aryl-(C_1-C_6)alkyl (wherein the alkyl moiety is linear or branched), a linear or branched (C_1-C_6)alkylene chain substituted by a group selected from -ORa and -NRaRb wherein Ra

and Rb are as defined hereinbefore,

- n represents an integer selected from 1 and 2,

and in the case where only one of the two groups R_5 and R_6 represents a group as defined hereinbefore then the other of the said groups R_5 or R_6 represents a group selected from a hydrogen atom, hydroxy, linear or branched (C_1-C_6)alkoxy, linear or branched (C_1-C_6)alkyl-carbonyloxy, arylcarbonyloxy, aryl-(C_1-C_6)alkyl-carbonyloxy (wherein the alkyl moiety is linear or branched), and amino optionally substituted by one or two, identical or different, linear or branched (C_1-C_6)alkyl groups,

* **R_7** represents a group selected from hydroxy, linear or branched (C_1-C_6)alkoxy, -C(W_2)-T_1, -W_1-C(W_2)-W_3-T_1, -W_1-C(W_2)-T_1, -W_1-S(O)$_n$-W_3-T_1 and-W_1-S(O)$_n$-T_1 wherein W_1, W_2, W_3, T_1 and n are as defined hereinbefore, or R_7 may have the additional meaning of a hydrogen atom when R_2 represents a group -O-T_2-ORa and/or when X represents a hydrogen atom and Y, located in the 13-position of the naphthyl system of the pentacyclic skeleton, represents an amino group optionally substituted by one or two identical or different groups selected independently of one another from linear or branched (C_1-C_6)alkyl, linear or branched (C_1-C_6)acyl and linear or branched trihalo-(C_1-C_6)alkyl-carbonyl,

* **R_8** represents a linear or branched (C_1-C_6)alkoxy or linear or branched (C_1-C_6)alkyl-carbonyloxy group, or may have the additional meaning of hydroxy when R_2 represents a group -O-T_2-ORa as defined hereinbefore,

to their enantiomers, diastereoisomers and N-oxides, and to addition salts thereof with a pharmaceutically acceptable acid or base.

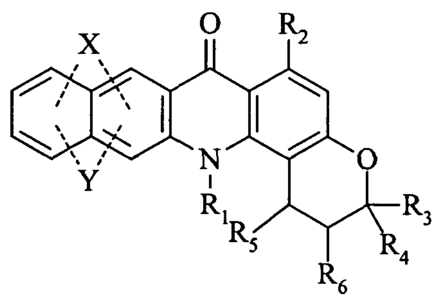
Aryl is understood to mean a phenyl or naphthyl group optionally containing one or more, identical or different, substitutents selected from hydroxy, halogen, carboxy, nitro, amino, linear or branched (C_1-C_6)alkylamino, di(C_1-C_6)alkylamino wherein each alkyl moiety may be linear or branched, linear or branched (C_1-C_6)alkoxy, linear or branched (C_1-C_6)acyl and linear or branched (C_1-C_6)alkyl-carbonyloxy.

Isomers are understood to comprise optical isomers, that is to say enantiomers and diastereoisomers.

Among the pharmaceutically acceptable acids there may be mentioned, without implying any limitation, hydrochloric acid, hydrobromic acid, sulphuric acid, phosphonic acid, acetic acid, trifluoroacetic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, glutaric acid, fumaric acid, tartaric acid, maleic acid, citric acid, ascorbic acid, oxalic acid, methanesulphonic acid, camphoric acid, lysine etc..

Among the pharmaceutically acceptable bases there may be mentioned, without implying any limitation, sodium hydroxide, potassium hydroxide, triethylamine, tert-butylamine etc..

According to an advantageous embodiment of the invention, preferred compounds are compounds of formula (IA) :



(IA)

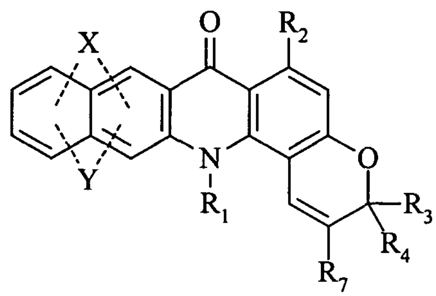
wherein X, Y, R_1, R_2, R_3, R_4, R_5 and R_6 are as defined for formula (I).

Preferred compounds of formula (IA) are compounds wherein R_5 and R_6 are identical and each represent a group of formula $-W_1-C(W_2)-W_3-T_1$ or $-W_1-S(O)_n-T_1$ wherein W_1, W_2, W_3, T_1 and n are as defined for formula (I).

In especially interesting manner, preferred compounds of formula (IA) are compounds wherein R_5 and R_6 are identical and each represent a group of formula $-W_1-C(W_2)-W_3-T_1$ wherein W_1 represents an oxygen atom, W_2 represents an oxygen atom, W_3 represents a nitrogen atom substituted by a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched and T_1 is as defined for formula (I).

In another especially interesting manner, preferred compounds of formula (IA) are compounds wherein R_5 and R_6 are identical and each represent a group of formula $-W_1-S(O)_n-T_1$ wherein W_1 represents an oxygen atom, T_1 is as defined for formula (I) and n is equal to 2.

According to a second advantageous embodiment of the invention, preferred compounds are compounds of formula (IB) :



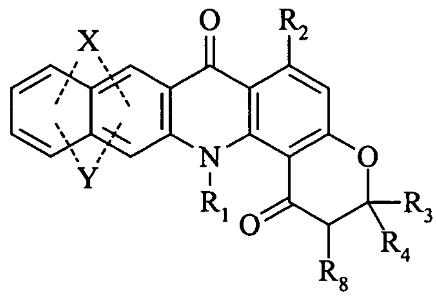
(IB)

wherein X, Y, R_1, R_2, R_3, R_4 and R_7 are as defined for formula (I).

Preferred compounds of formula (IB) are compounds wherein R_7 represents a group selected from $-C(W_2)-T_1$ and $-W_1-C(W_2)-T_1$ wherein W_1, W_2 and T_1 are as defined for formula (I).

In especially interesting manner, preferred compounds of formula (IB) are compounds wherein W_1 represents an oxygen atom, W_2 represents an oxygen atom and T_1 represents a linear or branched (C_1-C_6)alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched.

According to a third advantageous embodiment of the invention, preferred compounds are compounds of formula (IC) :



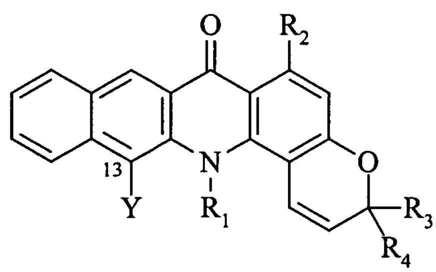
(IC)

wherein X, Y, R_1, R_2, R_3, R_4 and R_8 are as defined for formula (I).

Substituents R_3 and R_4 that are preferred according to the invention are linear or branched (C_1-C_6)alkyl groups.

Substituents R_2 that are preferred according to the invention are groups selected from linear or branched (C_1-C_6)alkoxy, -NRaRb, -O-T_2-NRaRb, -O-T_2-ORa, -NRa-T_2-NRaRb and -NRa-T_2-ORa wherein Ra, Rb, T_1 and T_2 are as defined for formula (I).

According to a fourth advantageous embodiment of the invention, preferred compounds are compounds of formula (IB_1) :



(IB_1)

wherein R_1, R_2, R_3 and R_4 are as defined for formula (IB) and Y represents an amino group optionally substituted by one or two identical or different groups selected independently of one another from linear or branched (C_1-C_6)alkyl, linear or branched (C_1-C_6)acyl and linear or branched trihalo-(C_1-C_6)alkyl-carbonyl.

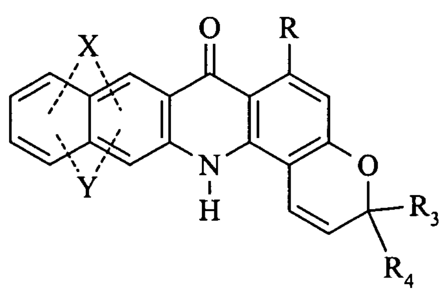
In especially advantageous manner, preferred compounds of the invention are:

- (1*S*,2*S*)-1-{[(dimethylamino)carbonyl]oxy}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl dimethylcarbamate,
- (1*S*,2*S*)-6-methoxy-3,3,14-trimethyl-2-{[(4-methylphenyl)sulphonyl]oxy}-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-1-yl 4-methylbenzenesulphonate,

- 6-methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl acetate,
- 2-benzoyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one
- 2-butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one,
- 2-acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one,
- 6-methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl butyrate,
- 6-(2-hydroxyethoxy)-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one,
- 13-amino-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-7-one,
- *N*-(6-methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-13-yl)acetamide,
- 6-methoxy-3,3-dimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]-acridin-2-yl acetate.

The enantiomers, diastereoisomers, N-oxides and addition salts with a pharmaceutically acceptable acid or base of the preferred compounds form an integral part of the invention.

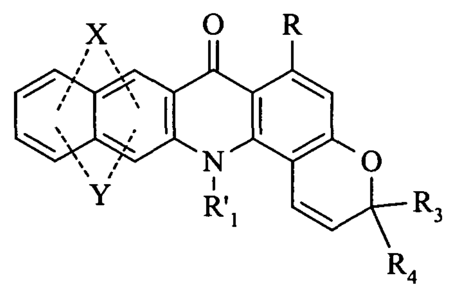
The present invention relates also to a process for the preparation of compounds of formula (I), which process is characterised in that there is used as starting material the compound of formula (VI) :



(VI),

wherein X, Y, R, R_3 and R_4 are as defined hereinbefore,

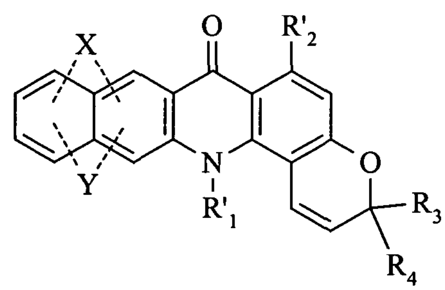
the nitrogen atom of which compound of formula (VI) is optionally substituted, by the action of an alkyl halide or of a dialkyl sulphate in the presence of a deprotonating agent, in an aprotic polar solvent or under phase transfer conditions, yielding the compounds of formula (IX) :



(IX),

wherein X, Y, R, R_3 and R_4 are as defined hereinbefore and R'_1 represents a linear or branched $(C_1\text{-}C_6)$alkyl group,

which compounds of formula (IX) are subjected to the action of an alkylating agent or an acylating agent under customary conditions of organic synthesis to yield the compounds of formula (X) :



(X),

wherein X, Y, R'_1, R_3 and R_4 are as defined hereinbefore and R'_2 represents a group selected from -ORa, -O-C(O)-T_1, -O-T_2-NRaRb and -O-T_2-ORa wherein Ra, Rb, T_1 and T_2 are as defined for formula (I),

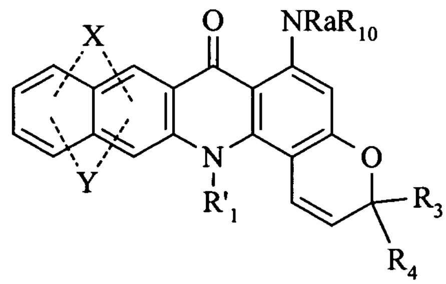
which compounds of formula (X), in the case where R'_2 represents an alkoxy group, are treated with a compound of formula (XI) :

$$HNRaR_{10} \qquad \textbf{(XI)},$$

wherein Ra represents a hydrogen atom, a linear or branched $(C_1\text{-}C_6)$alkyl group, an aryl group or an aryl-$(C_1\text{-}C_6)$alkyl group wherein the alkyl moiety is linear or branched, and R_{10} represents a group selected from Rb, -C(O)-T_1, -T_2-NRaRb, -T_2-ORa and -T_2-CO_2Ra

wherein Ra, Rb, T_1 and T_2 are as defined for formula (I),

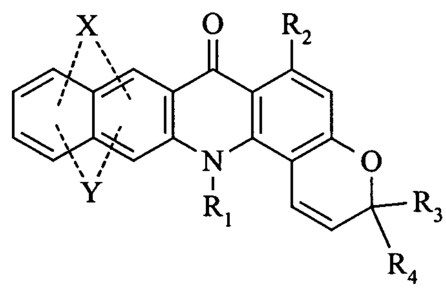
to yield the compounds of formula (XII) :



(XII),

wherein X, Y, R'_1, R_3, R_4, Ra and R_{10} are as defined hereinbefore,

the totality of the compounds of formulae (VI), (IX), (X) and (XII) forming the compounds of formula (XIII) :



(XIII),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined in the general definition for formula (I),

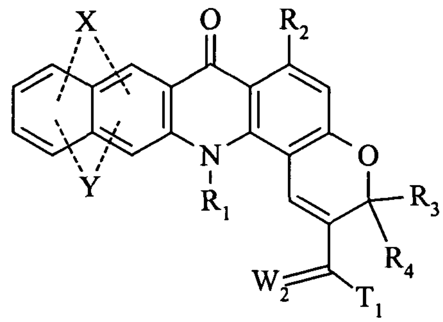
which compounds of formula (XIII) are subjected :

a) ⇨either to the action of a compound of formula (XIV) :

Hal-C(W_2)-T_1 **(XIV),**

wherein Hal represents a halogen atom and W_2 and T_1 are as defined in the general definition for formula (I),

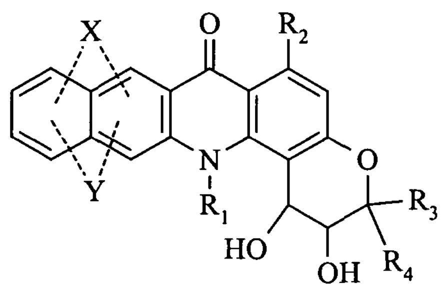
to yield the compounds of formula (I/a), a particular case of the compounds of formula (I) :



(I/a),

wherein X, Y, R_1, R_2, R_3, R_4, W_2 and T_1 are as defined hereinbefore,

b) ⇨or to the action of an oxidising agent for alkene functions to yield the compounds of formula (XV) :



(XV),

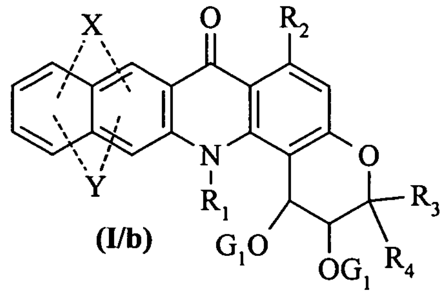
wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

which compounds of formula (XV) are treated with a compound of formula (XVI) :

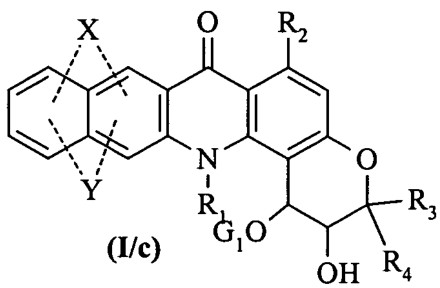
Hal-G_1 (XVI),

wherein Hal represents a halogen atom and G_1 represents a group selected from $-C(W_2)-W_3-T_1$, $-C(W_2)-T_1$, $-S(O)_n-W_3-T_1$ and $-S(O)_n-T_1$ wherein W_2, W_3, T_1 and n are as defined for formula (I),

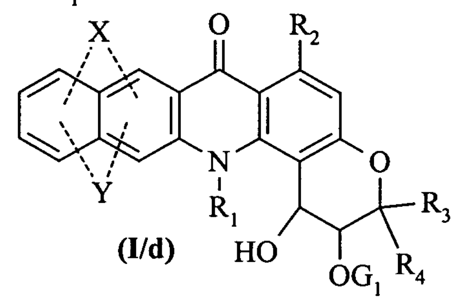
to yield the compounds of formulae (I/b), (I/c) and (I/d), particular cases of the compounds of formula (I) :



(I/b)



(I/c)



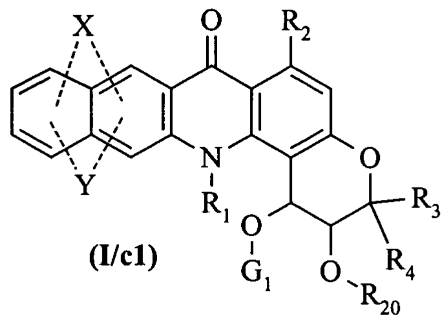
(I/d)

,

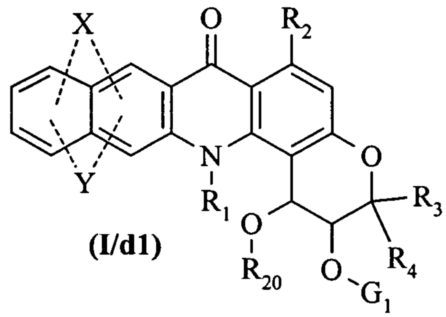
wherein X, Y, R_1, R_2, R_3, R_4 and G_1 are as defined hereinbefore,

which compounds of formulae (I/c) and/or (I/d) are subjected :

❖ either to the action of an alcohol of formula R_{20}-OH, wherein R_{20} represents a linear or branched (C_1-C_6)alkyl group, to yield the compounds of formulae (I/c1) and (1/d1), respectively :



(I/c1)

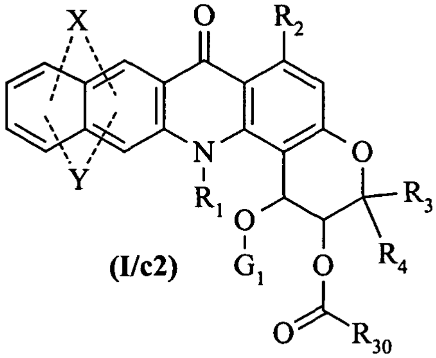


(I/d1)

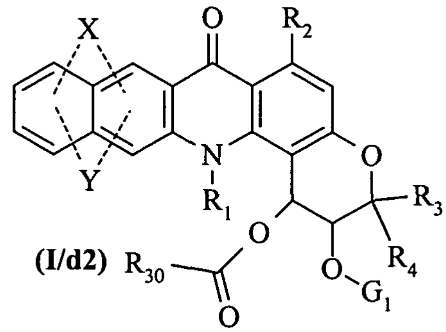
,

wherein X, Y, R_1, R_2, R_3, R_4, G_1 and R_{20} are as defined hereinbefore,

❖ or to the action of an anhydride of formula $(R_{30}CO)_2O$, wherein R_{30} represents a linear or branched (C_1-C_6)alkyl group, an aryl group or an aryl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched, to yield the compounds of formulae (I/c2) and (I/d2), respectively :



(I/c2)

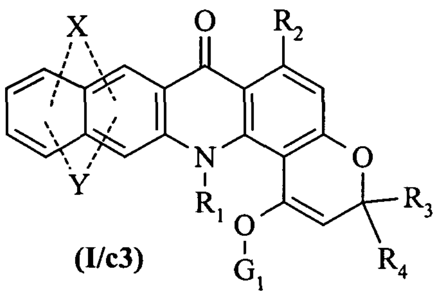


(I/d2)

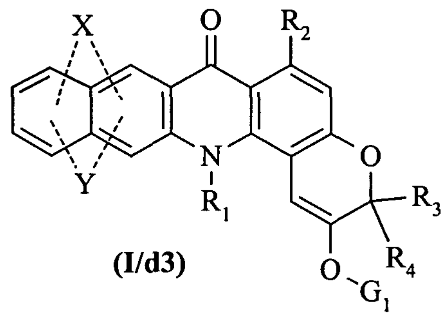
,

wherein X, Y, R_1, R_2, R_3, R_4, G_1 and R_{30} are as defined hereinbefore,

❖ or to dehydrating conditions in an acid medium to yield the compounds of formulae (I/c3) and (I/d3), respectively, particular cases of the compounds of formula (I) :



(I/c3)

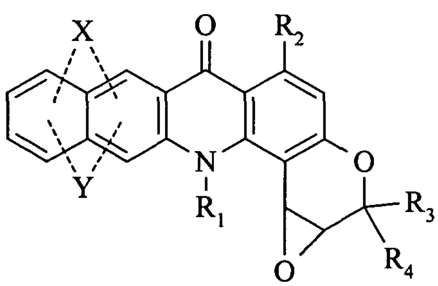


(I/d3)

,

wherein X, Y, R_1, R_2, R_3, R_4 and G_1 are as defined hereinbefore,

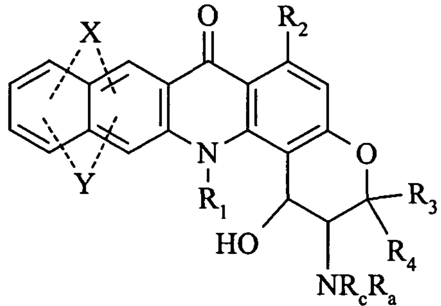
c) ⇨ or to the action of a peracid or of dimethyl dioxirane to yield the compounds of formula (XVII) :



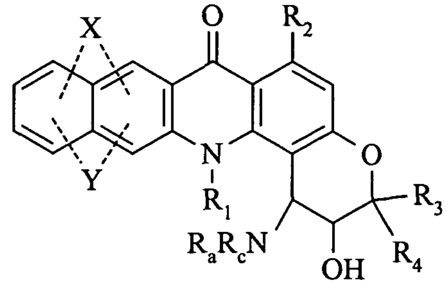
(XVII),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

which compounds of formula (XVII) are treated with ammoniac or with a primary amine to yield the compounds of formulae (XVIII/a) and/or (XVIII/b) :



(XVIII/a)

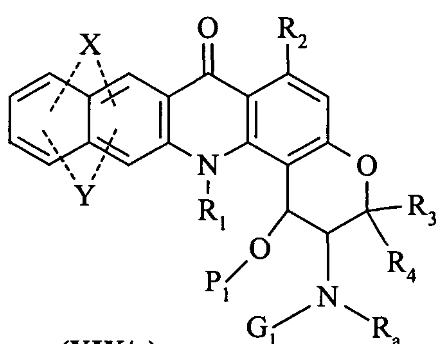


(XVIII/b)

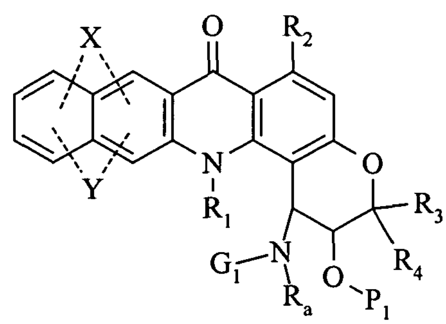
,

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore, R_c represents a hydrogen atom and R_a represents a hydrogen atom in the case where the reagent used is ammoniac or represents a group selected from linear or branched (C_1-C_6)alkyl, aryl and aryl-(C_1-C_6)alkyl (wherein the alkyl moiety is linear or branched) in the case where the reagent used is a primary amine,

the alcohol function of which compounds of formulae (XVIII/a) and (XVIII/b) is protected by a protecting group for hydroxy groups, the compounds then being subjected to the action of a compound of formula Hal-G_1 (XVI) as defined hereinbefore to yield the compounds of formulae (XIX/a) and (XIX/b), respectively,



(XIX/a)

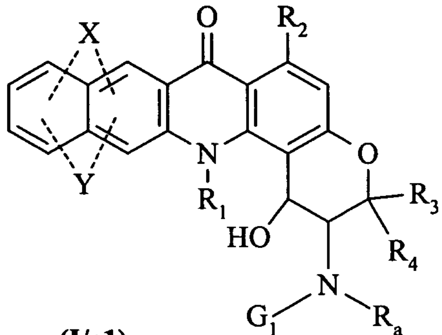


(XIX/b)

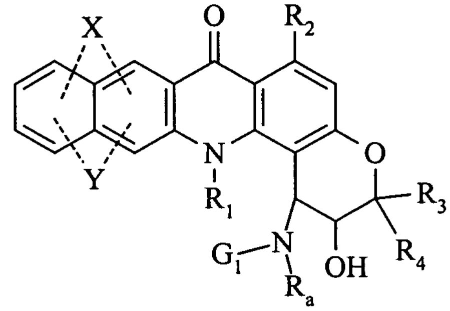
,

wherein X, Y, R_1, R_2, R_3, R_4, R_a and G_1 are as defined hereinbefore and P_1 represents a protecting group for hydroxy functions,

the hydroxy group of which compounds of formulae (XIX/a) and (XIX/b) is deprotected to yield the compounds of formulae (I/e1) and (I/f1), respectively, particular cases of the compounds of formula (I) :



(I/e1)



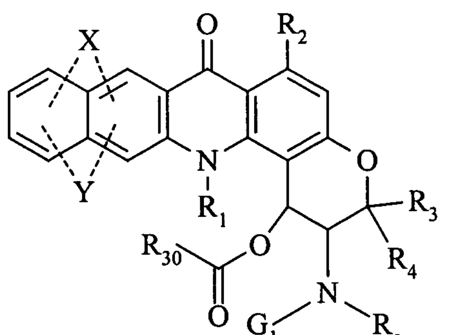
(I/f1)

,

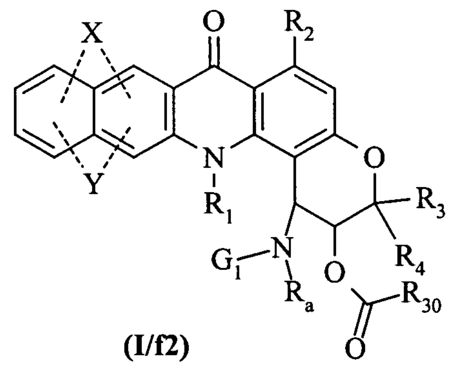
wherein X, Y, R_1, R_2, R_3, R_4, R_a and G_1 are as defined hereinbefore,

which compounds of formulae (I/e1) and (I/f1) are subjected :

❖ either to the action of an anhydride of formula $(R_{30}CO)_2O$, wherein R_{30} represents a linear or branched $(C_1\text{-}C_6)$alkyl group, an aryl group or an aryl-$(C_1\text{-}C_6)$alkyl group wherein the alkyl moiety is linear or branched, to yield the compounds of formulae (I/e2) and (I/f2), respectively, particular cases of the compounds of formula (I) :



(I/e2)

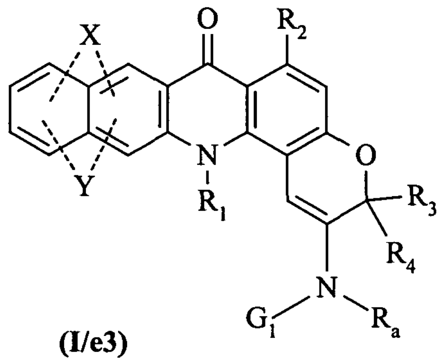


(I/f2)

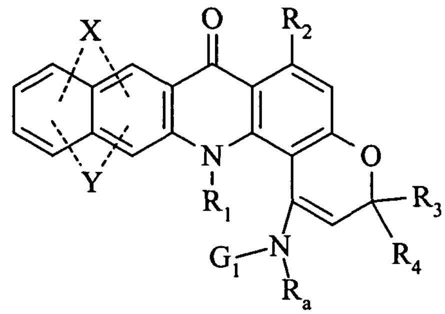
,

wherein X, Y, R_1, R_2, R_3, R_4, R_a, G_1 and R_{30} are as defined hereinbefore,

❖ or to dehydrating conditions in an acid medium to yield the compounds of formulae (I/e3) and (I/f3), respectively, particular cases of the compounds of formula (I) :



(I/e3)

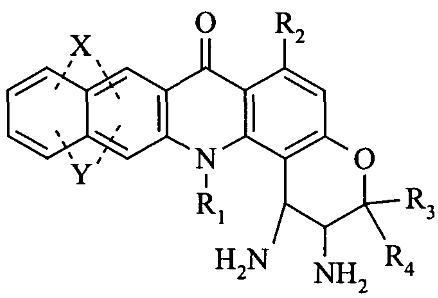


(I/f3)

,

wherein X, Y, R_1, R_2, R_3, R_4, R_a and G_1 are as defined hereinbefore,

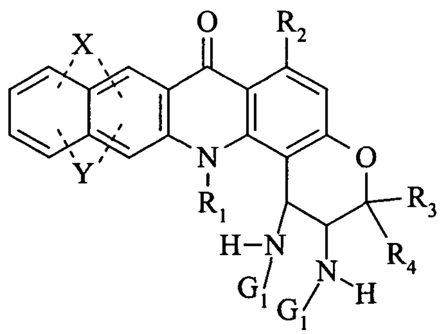
d) ⇨ or to the action of NaN_3 in the presence of hydrogen peroxide, followed by a reduction step using tri-n-butyltin hydride, for example, to yield the compounds of formula (XX) :



(XX),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

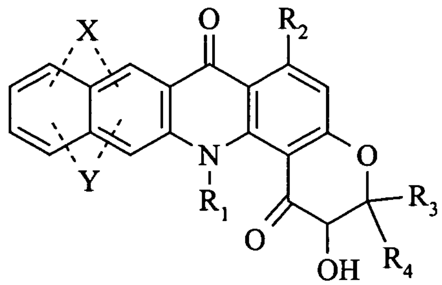
which compounds of formula (XX) are subjected to the action of a compound of formula Hal-G_1 (XVI) as defined hereinbefore to yield the compounds of formula (I/g), a particular case of the compounds of formula (I) :



(I/g),

wherein X, Y, R_1, R_2, R_3, R_4 and G_1 are as defined hereinbefore,

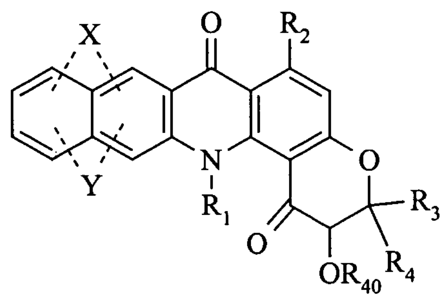
e) ⇨or to the action of potassium permanganate in a polar medium to yield the compounds of formula (XXI) :



(XXI),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

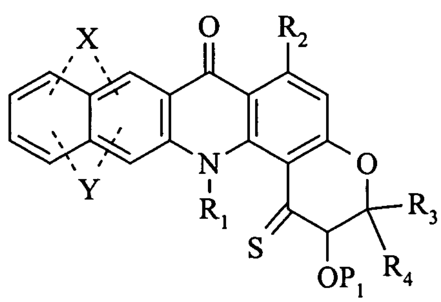
which compounds of formula (XXI) are subjected to the action of either an alkylating agent or an acylating agent to yield the compounds of formula (I/h), a particular case of the compounds of formula (I) :



(I/h),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore and R_{40} represents a linear or branched (C_1-C_6)alkyl group or a linear or branched (C_1-C_6)acyl group,

or the hydroxy functions of which compounds of formula (XXI) are protected with a protecting group conventionally used in organic synthesis, the compounds then being subjected to the action of P_2S_5 to yield the compounds of formula (XXII) :

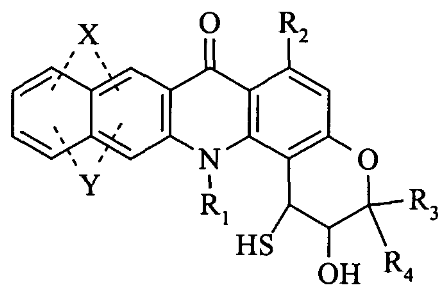


(XXII),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore and P_1 represents a protecting

group for hydroxy functions,

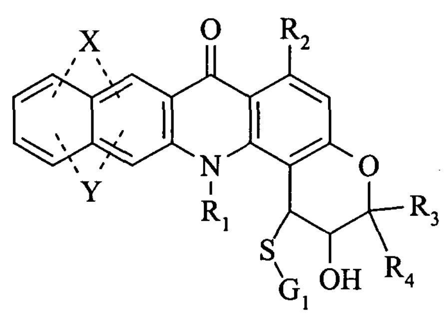
which compounds of formula (XXII) are treated with a reducing agent and then subjected to a reaction for deprotection of the hydroxy function to yield the compounds of formula (XXIII) :



(XXIII),

wherein X, Y, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

which compounds of formula (XXIII) are treated with a compound of formula (XVI) as defined hereinbefore to yield the compounds of formula (I/i), a particular case of the compounds of formula (I) :



(I/i),

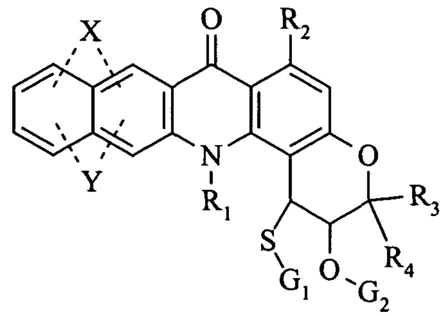
wherein X, Y, G_1, R_1, R_2, R_3 and R_4 are as defined hereinbefore,

which compounds of formula (I/i) may be subjected to the action of an alkylating agent, an acylating agent or a compound of formula (XVIa) :

Hal-G'$_1$ (XVIa),

wherein Hal represents a halogen atom and G'_1 represents a group selected from -C(W_2)-W_3-T_1, -C(W_2)-T_1, -S(O)$_n$-W_3-T_1 and -S(O)$_n$-T_1, wherein W_2, W_3, T_1 and n are as defined for formula (I),

to yield the compounds of formula (I/j), a particular case of the compounds of formula (I) :



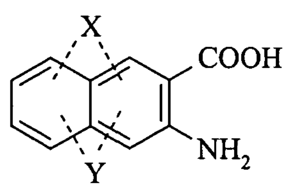
(I/j),

wherein X, Y, G_1, R_1, R_2, R_3 and R_4 are as defined hereinbefore and G_2 represents a group selected from G'_1 as defined hereinbefore, linear or branched $(C_1\text{-}C_6)$alkoxy, linear or branched $(C_1\text{-}C_6)$alkyl-carbonyloxy, arylcarbonyloxy and aryl-$(C_1\text{-}C_6)$alkyl-carbonyloxy (wherein the alkyl moiety is linear or branched),

the compounds of formulae (I/a) to (I/j), (I/c_1) to (I/c_3), (I/d_1) to (I/d_3), (I/e_1) to (I/e_3) and (I/f_1) to (I/f_3) constituting the totality of the compounds of the invention, which are purified, if necessary, according to a conventional purification technique, which may be, if desired, separated into their different isomers according to a conventional separation technique and which are converted, if desired, into their N-oxides and, where appropriate, their addition salts with a pharmaceutically acceptable acid or base.

The compounds of formula (VI) may advantageously be obtained :

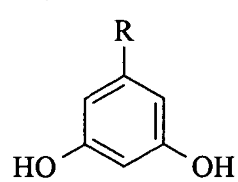
• *either starting from 3-amino-2-naphthalenecarboxylic acid compounds (II) :*



(II),

wherein X and Y are as defined for formula (I),

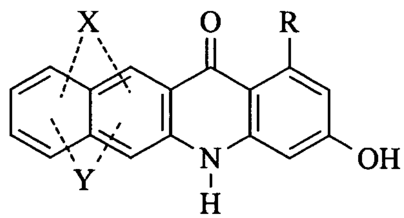
which are reacted with a phloroglucinol compound of formula (III) :



(III),

wherein R represents a hydrogen atom, a hydroxy group or a linear or branched $(C_1\text{-}C_6)$alkyl group,

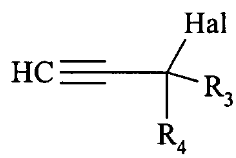
to yield the compounds of formula (IV) :



(IV),

wherein X, Y and R are as defined hereinbefore,

which are then treated under basic conditions in an aprotic solvent with an alkyne of formula (V) :

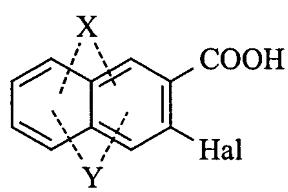


(V),

wherein Hal represents a halogen atom and R_3 and R_4 are as defined for formula (I),

to yield the compounds of formula (VI) as defined hereinbefore,

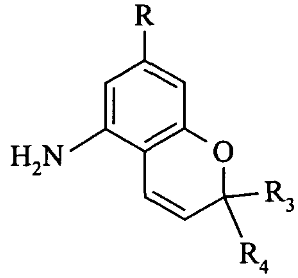
• *or starting from 3-halo-2-naphthalenecarboxylic acid compounds of formula (VII) :*



(VII),

wherein X and Y are as defined for formula (I) and Hal represents a halogen atom,

which are reacted with an amino-chromene compound of formula (VIII) :



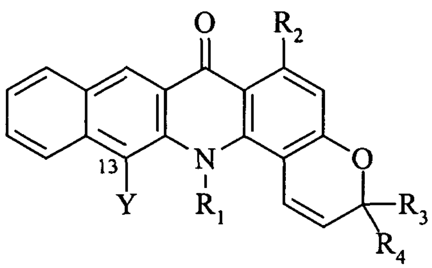
(VIII),

wherein R_3 and R_4 are as defined for formula (I) and R is as defined hereinbefore,

to yield, likewise, the compounds of formula (VI) as defined hereinbefore.

The compounds of formula (XIII) are synthesis intermediates that are useful in obtaining the compounds of formula (I). Among those compounds of formula (XIII) there are distinguished :

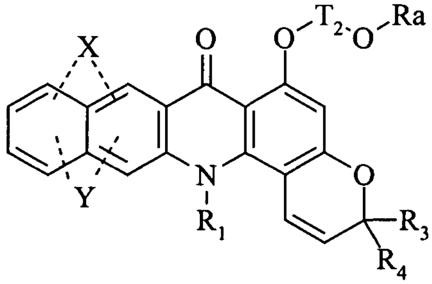
- compounds of formula (IB_1) :



(IB_1),

wherein R_1, R_2, R_3 and R_4 are as defined for formula (IB) and Y represents an amino group optionally substituted by one or two identical or different groups selected independently of one another from linear or branched (C_1-C_6)alkyl, linear or branched (C_1-C_6)acyl and linear or branched trihalo-(C_1-C_6)alkyl-carbonyl,

- and compounds of formula (IB_2) :



(IB_2),

wherein R_1, R_3, R_4, T_2, Ra, X and Y are as defined for formula (I).

Those compounds of formulae (IB_1) and (IB_2) are new and have cytotoxic activity. They are therefore useful in the treatment of cancers.

The compounds of formula (XXI) likewise are synthesis intermediates that are useful in obtaining the compounds of formula (I) and, more especially, compounds of formula (IC). Those compounds of formula (XXI) likewise have cytotoxic activity. Their use as an active ingredient in a pharmaceutical composition makes the latter useful in the treatment of cancers.

The compounds of formulae (II), (III), (V), (VII), (VIII), (XI), (XIV) and (XVI) either are commercially available compounds or are obtained according to conventional methods of organic synthesis well known to the person skilled in the art.

The compounds of formula (I) have especially valuable anti-tumour properties. They have excellent *in vitro* cytotoxicity with respect to cell lines originating from murine and human tumours, by virtue of specific blockage of the cell cycle, and are active *in vivo*, in the mouse, with respect to transplantable murine and human tumours. The characteristic properties of these compounds allow them to be used therapeutically as anti-tumour agents, especially for treatment of ovarian and lung carcinomas.

The present invention relates also to pharmaceutical compositions comprising, as active ingredient, at least one compound of formula (I), an enantiomer or diastereoisomer thereof, or an N-oxide thereof, or an addition salt thereof with a pharmaceutically acceptable acid or base, alone or in combination with one or more inert, non-toxic, pharmaceutically acceptable excipients or carriers.

Among the pharmaceutical compositions according to the invention there may be mentioned more especially those that are suitable for oral, parenteral (intravenous, intramuscular or subcutaneous), per- or trans-cutaneous, intravaginal, rectal, nasal, perlingual, buccal, ocular or respiratory administration.

Pharmaceutical compositions according to the invention for parenteral injections especially include aqueous and non-aqueous sterile solutions, dispersions, suspensions or emulsions and also sterile powders for reconstituting injectable solutions or dispersions.

Pharmaceutical compositions according to the invention for solid oral administrations especially include tablets or dragées, sublingual tablets, sachets, gelatin capsules and granules, and for liquid oral, nasal, buccal or ocular administrations especially include emulsions, solutions, suspensions, drops, syrups and aerosols.

Pharmaceutical compositions for rectal or vaginal administration are preferably suppositories and those for per- or trans-cutaneous administration especially include powders, aerosols, creams, ointments, gels and patches.

The pharmaceutical compositions mentioned hereinbefore illustrate the invention but do not limit it in any way.

Among the inert, non-toxic, pharmaceutically acceptable excipients or carriers there may be mentioned, by way of non-limiting example, diluents, solvents, preservatives, wetting agents, emulsifiers, dispersing agents, binders, swelling agents, disintegrating agents, retardants, lubricants, absorbents, suspending agents, colourants, aromatising agents etc..

The useful dosage varies according to the age and weight of the patient, the administration route, the pharmaceutical composition used, the nature and severity of the disorder and the administration of any associated treatments. The dosage ranges from 0.1 mg to 1000 mg per day in one or more administrations.

The Examples that follow illustrate the invention but do not limit it in any way.

The starting materials used are products that are known or that are prepared according to known operating procedures. The various Preparations yield synthesis intermediates that are useful in preparation of the compounds of the invention.

The structures of the compounds described in the Examples and Preparations were determined according to the usual spectrophotometric techniques (infrared, nuclear magnetic resonance, mass spectrometry, ...).

The melting points were determined using either a Kofler hot-plate or a hot-plate under a microscope. When the compound is in the form of a salt, the melting point given refers to the salt form of the compound.

<u>Preparation 1</u> : 6-Methoxy-3,3,14-trimethyl-7,14-dihydro-3*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-7-one

<u>Step A</u> : 1,3-Dihydroxy-5,12-dihydro-benzo[b]acridin-12-one

To a solution of 5 g of 3-amino-2-naphthalenecarboxylic acid in 50 ml of heptan-1-ol there are added 3.5 g of 1,3,5-trihydroxybenzene and 62.5 mg of para-toluenesulphonic acid. The mixture is stirred for 48 hours under reflux using a Dean-Stark apparatus, and the reaction mixture is then concentrated *in vacuo*. The residue is chromatographed over silica gel (eluant : cyclohexane/acetone : 90/10). The isolated product is crystallised from a cyclohexane/acetone mixture, allowing 5.2 g of the expected product to be obtained.

<u>Step B</u> : 6-Hydroxy-3,3-dimethyl-7,14-dihydro-3H-benzo[b]pyrano[3,2-h]acridin-7-one

To a solution of 2 g of the product of Step A in 50 ml of anhydrous dimethylformamide, under an inert atmosphere, there are added 2 g of anhydrous potassium carbonate. After stirring for 15 minutes at 65°C, 2.4 g of anhydrous potassium iodide and 4.4 g of 3-chloro-3-methyl-1-butyne are added, and the reaction mixture is held at 65°C for 24 hours and then at 130°C for 1 hour. After cooling, the solution is hydrolysed and then extracted with dichloromethane. The combined organic phases are washed with water and then with 1M potassium hydroxide solution, dried over sodium sulphate and then evaporated. After chromatography over silica gel (cyclohexane/acetone : 90/10), 1.10 g of the expected product are isolated.

Melting point : 225°C

Step C : *6-Methoxy-3,3,14-trimethyl-7,14-dihydro-3H-benzo[b]pyrano-[3,2-h]acridin-7-one*

To a solution of 0.5 g of the product obtained in Step B in 20 ml of anhydrous dimethylformamide there are slowly added, at 0°C, under an inert atmosphere, 0.16 g of sodium hydride and then, after 15 minutes, 0.65 ml of dimethyl sulphate (6 equivalents). After 1 hour, the reaction mixture is hydrolysed using ice and is then extracted with ethyl acetate. After washing the organic phase with aqueous sodium hydroxide solution, it is dried over sodium sulphate and then evaporated *in vacuo*. Chromatography over silica gel (cyclohexane/acetone : 98/2) allows 0.42 g of the expected product to be obtained.
Melting point : 188°C.

Preparation 2 : (±)-*cis*-1,2-Dihydroxy-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetra-hydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

To a solution of 2 g of the product of Preparation 1 and 0.9 g of 4-methylmorpholine-N-oxide monohydrate in 40 ml of a mixture of tert-butanol/tetrahydrofuran/water (10/3/1) there is added osmium tetroxide in the form of a 2.5 % solution in 3.8 ml of 2-methyl-2-propanol. After 2 days at ambient temperature, 105 ml of saturated $NaHSO_3$ solution are added, and the mixture is stirred for 1 hour and then extracted with dichloromethane. The combined organic phases are dried over sodium sulphate and concentrated *in vacuo*. Chromatography over silica gel (dichloromethane/methanol : 95/5) allows 1.3 g of the expected product to be isolated.
Melting point : 194°C.

Preparation 3 : 6-(Diethylaminopropylamino)-3,3,14-trimethyl-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

To 0.15 g of the product of Preparation 1 there are added 4 ml of N,N-diethyl-propylenediamine. After reacting for 5 days at 70°C under an inert atmosphere, the

reaction mixture is evaporated under reduced pressure. The residue obtained is chromatographed over silica gel (cyclohexane/ethyl acetate : 80/20), allowing the expected product to be isolated.

Mass spectrum : (DIC/NH_3) : m/z : 470 $(M+H)^+$

Preparation 4 : 6-[(2-Morpholin-4-yl)ethylamino]-3,3,14-trimethyl-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

The procedure is as in Preparation 3, using 4-(2-aminoethyl)-morpholine as reagent.

Mass spectrum : (DIC/NH_3) : m/z : 470 $(M+H)^+$

Preparation 5 : (±)-*cis*-1,2-Dihydroxy-6-(diethylaminopropylamino)-3,3,14-trimethyl-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7one

The procedure is as in Preparation 2, using the compound of Preparation 3 as substrate.

Preparation 6 : (±)-1-Amino-2-hydroxy-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

Step A : (±)-1-Azido-2-hydroxy-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetrahydro-1H-benzo[b]pyrano[3,2-h]acridin-7-one

To a solution of 0.15 g of the compound of Preparation 2 and 0.5 g of NaN_3 in 6 ml of chloroform there are slowly added, at ambient temperature, 3 ml of trifluoroacetic acid. After stirring for 12 hours, 1 equivalent of NaN_3 is added and the reaction mixture is held at ambient temperature for a further 12 hours. The reaction mixture is then washed with water and then with saturated NaCl solution and is dried over sodium sulphate. Chromatography over silica gel (dichloromethane/methanol : 95/5) allows the expected product to be isolated.

Step B : (±)-1-Amino-2-hydroxy-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetrahydro-1H-benzo[b]pyrano[3,2-h]acridin-7-one

A solution containing 0.2 g of the compound obtained in Step A and 0.09 g of Pd/C in 5 ml of ethanol is stirred at ambient temperature under an H_2 atmosphere for 48 hours. The catalyst is then filtered off and the filtrate is concentrated *in vacuo*. The residue is chromatographed over silica (dichloromethane/methanol : 95/5), allowing the desired product to be isolated.

Preparation 7 : 6-(Dimethylaminoethyloxy)-3,3,14-trimethyl-7,14-dihydro-3*H*-benzo-[*b*]pyrano[3,2-*h*]acridin-7-one

Step A : 6-Hydroxy-3,3,14-trimethyl-7,14-dihydro-3H-benzo[b]pyrano[3,2-h]acridin-7-one

The product is obtained according to the procedure of Preparation 1, using only 1.5 equivalents of sodium hydride and 2 equivalents of dimethyl sulphate.

Melting point : 138 °C.

Step B : 6-(Dimethylaminoethyloxy)-3,3,14-trimethyl-7,14-dihydro-3H-benzo[b]-pyrano[3,2-h]acridin-7-one

To a solution, under nitrogen, of 0.2 g of the compound obtained in Step A in 20 ml of dimethylformamide, there are added 1 equivalent of sodium hydride and 1 equivalent of 2-dimethylaminoethyl chloride hydrochloride. After 48 hours at 70°C, the reaction mixture is cooled; it is then poured onto 80 ml of ice-cold water and extracted with dichloromethane. After washing and drying over $MgSO_4$, the solution is evaporated under reduced pressure. Chromatography over silica gel (ethyl acetate/cyclohexane : 80/20) allows the expected product to be isolated.

Mass spectrum : (DIC/NH_3) : m/z : 429 $(M+H)^+$

Preparation 8 : 1,2-Diamino-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

Chromatography over silica gel allows isolation of this co-product, which forms during synthesis of the compound of Preparation 6.

<u>Example 1 : 6-(2-Hydroxyethoxy)-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one</u>

A solution of 500 mg of the compound of Preparation 1 in 5 ml of dichloroethane in the presence of 36 mg of $AlCl_3$ is heated at 70°C for 3 hours. The reaction mixture is then poured onto 10 % HCl solution and extracted with dichloromethane. After conventional treatment of the organic phases and evaporation thereof under reduced pressure, chromatography of the residue over silica gel (dichloromethane/methanol : gradient from 0.2 to 10 %) allows the expected product to be isolated.

<u>Melting point :</u> 230°C

<u>Example 2 : 2-Butyryl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one</u>

A mixture of 0.81 mmol of butyryl chloride and 0.673 mmol of $AlCl_3$ in 2 ml of anhydrous dichloromethane is added, in small portions, to 0.135 mmol of the product of Preparation 1 in 2 ml of dichloromethane at 0°C. The reaction mixture is stirred for 4 hours at ambient temperature and is then poured onto 10 % HCl solution. After conventional treatment of the organic phases and evaporation thereof under reduced pressure, chromatography of the residue over silica gel (dichloromethane/methanol : gradient from 0.1 to 6 %) allows the expected product to be isolated.

<u>Melting point :</u> 302°C

<u>Example 3 : 2-Benzoyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one</u>

The product is obtained according to the procedure of Example 2, using benzoyl chloride as reagent.

<u>Melting point :</u> 319°C

Example 4 : 2-Acetyl-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one

The product is obtained according to the procedure of Example 2, using acetyl chloride as reagent.

Melting point : 277°C

Example 5 : 2-Butyryl-6-(diethylaminopropylamino)-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*] acridin-7-one

The product is obtained according to the procedure of Example 2, using the compound of Preparation 3 as substrate.

Example 6 : 2-Butyryl-6-[(2-morpholin-4-yl)ethylamino]-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

The product is obtained according to the procedure of Example 2, using the compound of Preparation 4 as substrate.

Example 7 : (±)-cis-1,2-Dipentenoyloxy-6-methoxy-3,3,14-trimethyl-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

A solution of 1.48 mmol of the compound of Preparation 2 in 15 ml of anhydrous pyridine is added to 14.8 mmol of pentenoyl chloride. The reaction mixture is stirred at ambient temperature for 6 hours and is then extracted with dichloromethane. The combined organic phases are dried over magnesium sulphate, filtered and then evaporated under reduced pressure. Chromatography of the residue over silica gel (dichloromethane/methanol : gradient from 0.2 to 2 %) allows the expected product to be isolated.

Melting point : 152°C

Example 8 : (±)-cis-1-Hydroxy-6-methoxy-2-pentenoyloxy-3,3,14-trimethyl-2,3,7,14-

tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one

The co-product is isolated during chromatography of the compound of Example 7 over silica gel.

Melting point : 194°C

Example 9 : 6-Methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-2-yl butyrate

Step 1 : *(±)-cis-1-Hydroxy-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1H-benzo[b]pyrano[3,2-h]acridin-2-yl butyrate*

To a solution of 0.74 mmol of the compound of Preparation 2 in 7 ml of anhydrous pyridine in the presence of 4-dimethylaminopyridine there are added 2 equivalents of butyryl chloride. The reaction mixture is stirred at ambient temperature. After 72 hours, 5 equivalents of butyryl chloride are added and the mixture is stirred for 72 hours and then evaporated to dryness. Chromatography over silica gel allows the expected product to be isolated.

Melting point : 197°C

Step 2 : *6-Methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3H-benzo[b]pyrano-[3,2-h]acridin-2-yl butyrate*

To a solution of 0.29 mmol of the compound obtained in Step 1 in 6 ml of dichloromethane there are added 4 drops of 10 % HCl solution. The reaction mixture is stirred at ambient temperature for 3 days and is then dried and evaporated under reduced pressure. Chromatography of the residue over silica gel (dichloromethane/methanol : gradient from 0.2 to 5 %) allows the expected product to be isolated.

Melting point : 180°C

Example 10 : 6-Methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano-[3,2-*h*]acridin-2-yl acetate

Step 1 : *(±)-cis-1-Hydroxy-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1H-*

benzo[b]pyrano[3,2-h]acridin-2-yl acetate

The product is obtained according to the procedure of Step 1 of Example 9, using acetyl chloride as reagent.

<u>Step 2</u> : *6-Methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3H-benzo[b]pyrano-[3,2-h]acridin-2-yl acetate*

The product is obtained according to the procedure of Step 2 of Example 9, using the compound obtained in Step 1 above as substrate.

<u>Melting point</u> : 165°C

<u>Example 11 : (1S,2S)-1-{[(Dimethylamino)carbonyl]oxy}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl dimethylcarbamate</u>

In a flask, 0.698 mmol of potassium hydride is washed three times with hexane and then cooled to -10°C, and a solution of 0.123 mmol of the compound of Preparation 2 in 4 ml of anhydrous tetrahydrofuran is added. After the dropwise addition of 0.327 mmol of *N,N*-dimethylcarbamoyl chloride at -10°C, stirring is carried out for 3 hours 30 minutes at ambient temperature. The reaction is stopped by adding 50 ml of ethyl acetate and 10 ml of saturated $NaHCO_3$ solution in order to obtain a pH of 8. The organic phase is washed with water and dried over magnesium sulphate. After evaporation under reduced pressure and crystallisation from ethyl acetate, the expected product is isolated.

<u>Melting point</u> : 173°C

<u>Example 12 : (1S,2S)-6-Methoxy-3,3,14-trimethyl-2-{[(4-methylphenyl)sulphonyl]-oxy}-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-1-yl 4-methylbenzenesulfonate</u>

The product is obtained according to the procedure of Example 11, using tosyl chloride as reagent.

Example 13 : (1S,2S)-6-{[3-(Diethylamino)propyl]amino}-1-{[(dimethylamino)-carbonyl]oxy}-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl dimethylcarbamate

The product is obtained according to the procedure of Example 11, Steps 1 to 2, using the product of Preparation 5 as substrate.

Example 14 : (1S,2S)-1-{[(Dimethylamino)carbonyl]amino}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl dimethylcarbamate

The product is obtained according to the procedure of Example 11, Steps 1 to 2, using the product of Preparation 6 as substrate.

Example 15 : *N'*-((1S,2S)-1-{[(Dimethylamino)carbothioyl]amino}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl)-*N*,*N*-dimethylthiourea

The product is obtained according to the procedure of Example 11, Steps 1 to 2, using the product of Preparation 8 as substrate and dimethylthiocarbamoyl chloride as reagent.

Example 16 : 6-[2-(Dimethylamino)ethoxy]-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl butyrate

The product is obtained according to the procedure of Steps 1 to 2 of Example 9, using the compound of Preparation 7 as substrate in Step 1.

Example 17 : 13-Amino-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-7-one

Step 1 : *3-Acetamido-4-nitro-2-naphthalenecarboxylic acid*

A solution of 5 g of 3-amino-2-naphthalenecarboxylic acid in 40 ml of acetic anhydride is stirred at ambient temperature for 30 minutes and then 5 ml of nitric acid are added dropwise. The reaction mixture is then stirred in an ice bath for 7 days and is then poured onto ice. The precipitate formed is filtered off, washed with water and then dried and rinsed with 40 ml of methanol, allowing the expected product to be isolated after drying.
Melting point : *235°C*

Step 2 : *3-Amino-4-nitro-2-naphthalenecarboxylic acid*

A solution of 1 g of the compound obtained in Step 1 in 18 ml of hydrochloric acid is refluxed for 4 hours and then poured onto ice. The precipitate formed is filtered off, washed with water until the pH is 5-6 and then dried, allowing the expected product to be isolated.
Melting point : *238°C*

Step 3 : *3-Chloro-4-nitro-2-naphthalenecarboxylic acid*

1.2 g of sodium nitrite are slowly added to 13 ml of concentrated sulphuric acid; the temperature is brought to 70°C until the sodium nitrite has dissolved completely; the solution is then cooled to 20°C.
A solution of 3 g of the compound obtained in Step 2 above in 27 ml of acetic acid is cautiously added to the reaction mixture. The solution is then heated at 40°C for 30 minutes and is then cooled in an ice bath.
A solution of cuprous chloride (3.3 g) in 30 ml of concentrated hydrochloric acid is cooled in an ice bath and the solution of the diazoic compound previously obtained is slowly added. The temperature of the reaction mixture is then brought to 80°C and the reaction mixture is stirred until the evolution of gas has ceased.
Water is added to the reaction mixture, whilst cooling the latter in an ice bath. The precipitate formed is filtered off, carefully rinsed with water and dried. Crystallisation from dichloromethane allows the expected product to be obtained.

Melting point : 246°C

Step 4 : *3-[(2,2-Dimethyl-7-methoxy-2H-chromen-5-yl)amino]-4-nitro-2-naphthalene-carboxylic acid*

To 3.3 g of 5-amino-7-methoxy-2,2-dimethylchromene there are added 3 g of the compound obtained in Step 3 above, 3.4 g of potassium acetate, 375 mg of $Cu(OAc)_2.H_2O$ and 120 ml of 2-propanol containing 3.8 ml of triethylamine. The solution is refluxed for 5 days and is then evaporated under reduced pressure. The residue is taken up in a 1/1 mixture of dichloromethane and 1N aqueous HCl solution. After extraction of the aqueous phase, the combined organic phases are treated in customary manner. Chromatography over silica gel (dichloromethane/methanol : gradient from 0.1 to 1 %) allows the expected product to be isolated.
Melting point :165°C

Step 5 : *6-Methoxy-3,3-dimethyl-13-nitro-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]-acridin-7-one*

772 mg of the compound obtained in Step 4 are dissolved in 45 ml of anhydrous dichloromethane, and 3 ml of trifluoroacetic anhydride are added. After stirring for 5 minutes at ambient temperature, the reaction mixture is evaporated. The residue is taken up in a mixture of dichloromethane and saturated $NaHCO_3$ solution. After extraction of the aqueous phase with dichloromethane, the combined organic phases are treated in conventional manner and then distilled, allowing the expected product to be isolated.
Melting point : >350°C

Step 6 : *6-Methoxy-3,3,14-trimethyl-13-nitro-3,14-dihydro-7H-benzo[b]pyrano-[3,2-h]acridin-7-one*

To a solution of 300 mg of the compound obtained in Step 5 in 30 ml of dimethylformamide there are added 1.48 g of sodium hydride and then 1.8 ml of iodomethane. The reaction mixture is refluxed, under argon, for 3 days, and is then slowly

hydrolysed by the addition of ice. The precipitate formed is filtered off and then dried and is chromatographed over silica gel (dichloromethane/methanol : gradient from 0.1 to 0.5 %), allowing the expected product to be isolated.

<u>Melting point :</u> 283°C

<u>*Step 7 :*</u> *13-Amino-6-methoxy-3,3,14-trimethyl-3,14-dihydro-7H-benzo[b]pyrano-[3,2-h]acridin-7-one*

To 178 mg of the compound obtained in Step 6 above in 10 ml of acetic acid and 1 ml of water there are added 621 mg of zinc wool. The reaction mixture is stirred at ambient temperature for 2.5 hours and is then filtered. The filtrate is made alkaline using 10 % aqueous ammonium hydroxide solution; the precipitate formed is then separated off by filtration. The crude residue is purified by chromatography over silica gel (dichloromethane/methanol : gradient from 0.1 to 0.5 %), allowing the expected product to be isolated.

<u>Melting point :</u> 157°C

<u>Example 18 : 2,2,2-Trifluoro-*N*-(6-methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]pyrano[3,2-*h*]acridin-13-yl)acetamide</u>

A solution of 0.039 mmol of the compound obtained in Example 17 in 7 ml of anhydrous pyridine, under argon, is stirred at -20°C, and 0.15 mmol of trifluoroacetic anhydride is added. The reaction mixture is stirred for 6 hours at -20°C and is then diluted with 15 ml of methanol and concentrated under reduced pressure. Chromatography of the residue over silica gel (dichloromethane/methanol : 99.9/0.1) allows the expected product to be isolated.

<u>Example 19 : *N*-(6-Methoxy-3,3,14-trimethyl-7-oxo-7,14-dihydro-3*H*-benzo[*b*]-pyrano[3,2-*h*]acridin-13-yl)acetamide</u>

The product is obtained according to the procedure of Example 18, using acetic anhydride as reagent.

Example 20 : 6-Methoxy-3,3,14-trimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl acetate

Step 1 : *2-Hydroxy-6-methoxy-3,3,14-trimethyl-2,3-dihydro-1H-benzo[b]pyrano-[3,2-h]acridine-1,7(14H)-dione*

A suspension of 1.28 g of $KMnO_4$ in 15 ml of water is added dropwise, over 30 minutes, to a solution of 0.5 g of the product of Preparation 1 dissolved in 25 ml of acetone. The reaction mixture is stirred at ambient temperature for 8 hours and is then extracted with 2-butanone. The organic phase is treated in conventional manner and chromatography of the residue over silica gel (dichloromethane/methanol : 98/2) allows the expected product to be isolated.

Melting point : *272°C*

Step 2 : *6-Methoxy-3,3,14-trimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1H-benzo-[b]pyrano[3,2-h]acridin-2-yl acetate*

The product is obtained according to the procedure of Step 1 of Example 9, using acetic anhydride as reagent.

Melting point : *206°C*

Example 21 : 6-Methoxy-3,3,14-trimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1*H*-benzo-[*b*]pyrano[3,2-*h*]acridin-2-yl propionate

The product is obtained according to the procedure of Example 20, using propionic anhydride as reagent in Step 2.

Melting point : *218°C*

Example 22 : 6-Methoxy-3,3-dimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1*H*-benzo-[*b*]pyrano[3,2-*h*]acridin-2-yl acetate

Step 1 : *3-[(8-Methoxy-2,2-dimethyl-2H-chromen-5-yl)amino]-2-naphthoic acid*

A solution containing 1.2 mmol of 3-bromonaphthalene-2-carboxylic acid, 1.19 mmol of 7-methoxy-2,2-dimethyl-2*H*-1-benzopyran-5-ylamine, 2.38 mmol of potassium acetate, 10 mg of $Cu(OAc)_2.H_2O$ and 1.19 mmol of triethylamine in 17 ml of 2-propanol is refluxed for 24 hours and then concentrated under reduced pressure. The residue obtained is taken up in a 2/1 mixture of dichloromethane/1N HCl. The aqueous phase is extracted with dichloromethane and the combined organic phases are then treated in conventional manner. Chromatography of the residue over silica gel (dichloromethane) allows the expected product to be isolated.

<u>Step 2 :</u> *6-Methoxy-3,3-dimethyl-3,14-dihydro-7H-benzo[b]pyrano[3,2-h]acridin-7-one*

To a solution containing 1.94 mmol of the compound obtained in Step 1 in 20 ml of anhydrous dichloromethane, maintained at 0°C under argon, there are added 9.71 mmol of trifluoroacetic anhydride. After reacting for 3 days at ambient temperature, the reaction mixture is concentrated under reduced pressure. The residue is taken up in a mixture of dichloromethane and aqueous $NaHCO_3$ solution. After extraction with dichloromethane, the combined organic phases are treated in conventional manner. Chromatography of the residue over silica gel (dichloromethane) allows the expected product to be isolated, the latter being subsequently recrystallised from ethyl ether.

<u>Melting point :</u> *278°C*

<u>Step 3 :</u> *2-Hydroxy-6-methoxy-3,3-dimethyl-2,3-dihydro-1H-benzo[b]pyrano[3,2-h]-acridine-1,7(14H)-dione*

To a solution of 1.33 mmol of the compound obtained in Step 2 in 30 ml of a 10/3/1 mixture of *tert*-butanol/tetrahydrofuran/water there are added osmium tetroxide in the form of a 2.5 % solution in 0.67 ml of 2-methyl-2-propanol and 1.35 mmol of 4-methylmorpholine-N-oxide monohydrate. After stirring for two days at ambient temperature, $NaHSO_3$ solution is added. After stirring for one hour, the reaction mixture is extracted with dichloromethane. The organic phase is dried, filtered and then evaporated

under reduced pressure. Chromatography of the residue over silica gel (dichloromethane/methanol : 98/2) allows the expected product to be isolated.

<u>*Melting point :*</u> *230°C*

<u>*Step 4 :*</u> *6-Methoxy-3,3-dimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1H-benzo-[b]pyrano[3,2-h]acridin-2-yl acetate*

The product is obtained according to the procedure of Step 2 of Example 20, using an excess of acetic anhydride.

<u>*Melting point :*</u> *206°C*

<u>Example 23 : (±)-cis-1-{[(Diethylamino)carbonyl]oxy}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl diethylcarbamate</u>

The product is obtained according to the procedure of Example 11, using *N,N*-diethylcarbamoyl chloride as reagent.

<u>*Melting point :*</u> *193°C*

<u>Example 24 : (±)-cis-1-{[(Diisopropylamino)carbonyl]oxy}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl diisopropylcarbamate</u>

The product is obtained according to the procedure of Example 11, using *N,N*-diisopropylcarbamoyl chloride as reagent.

<u>*Melting point :*</u> *149.5°C*

<u>Example 25 : (±)-cis-1-{[(Dibutylamino)carbonyl]oxy}-6-methoxy-3,3,14-trimethyl-7-oxo-2,3,7,14-tetrahydro-1*H*-benzo[*b*]pyrano[3,2-*h*]acridin-2-yl dibutylcarbamate</u>

The product is obtained according to the procedure of Example 11, using *N,N*-

dibutylcarbamoyl chloride as reagent.

Melting point : 138°C

Example 26 : 6-Methoxy-3,3-dimethyl-1,7-dioxo-2,3,7,14-tetrahydro-1*H*-benzo-[*b*]pyrano[3,2-*h*]acridin-2-yl propionate

To a previously cooled solution of anhydrous pyridine (1.5 ml) and an excess of propionic anhydride there is added 0.26 mmol of the compound obtained in Step 3 of Example 22. The reaction mixture is stirred at ambient temperature, protected from light, for 24 hours and is then poured onto ice-cold water (10 ml). The precipitate obtained is filtered off, washed with water and dried under a phosphoric vacuum. Chromatography of the residue over silica gel (dichloromethane) allows the expected product to be isolated.

Melting point : 231°C

PHARMACOLOGICAL STUDY OF COMPOUNDS OF THE INVENTION

EXAMPLE 27 : *In vitro* activity

Murine leukaemia L1210 was used *in vitro*. The cells are cultured in RPMI 1640 complete culture medium containing 10 % foetal calf serum, 2 mM glutamine, 50 units/ml of penicillin, 50 µg/ml of streptomycin and 10 mM Hepes, pH: 7.4. The cells are distributed on microplates and exposed to the cytotoxic compounds for 4 doubling periods, or 48 hours. The number of viable cells is then quantified by means of a colorimetric assay, the Microculture Tetrazolium Assay (J. Carmichael *et al.*, Cancer Res., 47, 936-942, (1987)). The results are expressed as IC_{50}, the concentration of cytotoxic agent which inhibits the proliferation of the treated cells by 50 %.

By way of illustration, the compounds of Examples 11 and 7 exhibit an IC_{50} of 0.78 µM and 0.52 µM, respectively, thereby demonstrating their greater activity than the reference compound (acronycine).

EXAMPLE 28 : *In vitro* activity

1- Anti-tumour activity with respect to P388 leukaemia

Line P388 (murine leukaemia) was supplied by the National Cancer Institute (Frederick, USA). The tumour cells (10^6 cells) were inoculated on day 0 into the peritoneal cavity of female B6D2F1 mice (Iffa Credo, France). Six mice weighing from 18 to 20 g were used in each test group. The products were administered by the intravenous route on day 1. The anti-tumour activity is expressed as % T/C :

$$\% \text{ T/C (mouse)} = \frac{\text{Median survival time of the treated animals}}{\text{Median survival time of the control animals}} \times 100$$

The compounds of the present invention are very active in this model, having a T/C of > 150 %, whereas acronycine is only marginally active.

2- Anti-tumour activity with respect to C38 adenocarcinoma of the colon

Tumour fragments of C38 adenocarcinoma of the colon weighing approximately 30 mg were implanted under the skin of B6D2F1 mice (Iffa Credo, France) on day 0.

After growth of the tumour, the mice were divided into control (18 animals) and treated (6 or 7 animals) groups, which were homogeneous with respect to tumour size. The products were administered by the i.v. route once per week for 3 weeks (on days 10, 17 and 24), at their Maximum Tolerated Dose (MTD), MTD/2 and MTD/4.

The tumours were measured twice a week and the tumour volumes were calculated according to the following formula : volume (mm^3) = length (mm) x breadth (mm^2) / 2.

The anti-tumour activity is expressed as % T/C :

$$\% \text{ T/C} = \frac{\text{median Vt/V0 of the treated animals}}{\text{median Vt/V0 of the control animals}} \times 100$$

V0 and Vt being the initial volume of the tumour and its volume at measurement time t, respectively.

The optimum dose is the dose giving the lowest T/C value without toxicity (early death or weight loss greater than 20 %).

By way of example, the compounds of Examples 7 and 11 exhibit an anti-tumour activity of 30 % for an optimum dose of 12.5 mg/kg and 41 % for an optimum dose of 25 mg/kg, respectively, whereas acronycine exhibits an anti-tumour activity of 27 % for an optimum dose of 100 mg/kg, thereby demonstrating their strong therapeutic potential.

EXAMPLE 29

The compounds of the present invention having cytotoxic and anti-tumour activities, they will be used in the treatment of cancers. The compounds disclosed in the present application exhibit cytotoxic properties on a panel of murine and human cell lines, these results being predictive of the use of these compounds in the treatment of many tumours.

This is illustrated by the following test of cytotoxicity performed with the murine cell line L1210 and a human cell line KB-3-1, following the procedure described in Example 27

Results

Table 1 - Cytotoxicity on Murine L 1210 cells

Compounds	***IC_{50} (µM)***
Example 7	0.5
Example 8	0.9
Example 11	0.8
Example 23	0.9

The results disclosed in Table 1 show the effectiveness of more species compounds of the invention and demonstrate that compounds of the invention have utility in the treatment of tumours such as leukemia.

Table 2 - Cytotoxicity on Human KB-3-1 (bucal epidermoïde carcinoma) cell

Compounds	***IC_{50} (µM)***
Example 1	0.5
Example 7	0.14
Example 8	0.1
Example 11	0.4

Table 2 shows clearly that compounds of the invention are potently cytotoxic, consequently they can be used in the treatment of tumours such as carcinoma.

This point is supported by the results of Table 3, in which is presented in-vivo anti-tumours activities of the compounds of the invention on C38 colon adenocarcinoma, and two human solid tumors, A549 lung carcinoma and IGROV1 ovarian carcinoma.
The procedure used in the anti-tumour activity with respect to C38 adenocarcinoma is the same as the procedure described in the present application at Example 28.

The procedures used in the anti-tumour activity with respect to A549 lung carcinoma and IGROV1 ovarian carcinoma are described below.

Material and Methods

Anti-tumour activity on A549 lung carcinoma

The anti-tumour activity of the compounds against the human A549 non-small cell lung carcinoma was evaluated essentially as described (Kraus-Berthier, L., Jan, M., Guilbaud, N., Naze, M., Pierré, A., and Atassi, G. Histology and sensitivity to anticancer drugs of two human non-small cell lung carcinomas implanted in the pleural cavity of nude mice. Clinical Cancer Res., 6, 297-304, 2000). Briefly, 10^6 A549 cells were implanted through the chest wall into the left pleural space of nude mice in a volume of 100 μl. Compounds were administered i.v. at three doses on days 14 and 24 after tumor graft. Animal mortality was checked daily and the anti-tumour activity was expressed as T/C % = (median survival time of treated group/median survival time of control group) x 100. Results given in table 3 are the highest T/C values obtained at the maximum non-toxic dose, i.e. the optimal dose.

Anti-tumour activity on IGROV1 ovarian carcinoma

The anti-tumour activity of the compounds against the human IGROV1 ovarian carcinoma was evaluated essentially as described (Burbridge, M.F., Kraus-Berthier, L., Naze, M., Pierré, A., Atassi, G. and Guilbaud, N. Biological and pharmacological characterization of three models of human ovarian carcinoma established in nude mice : use of the CA125 tumor marker to predict antitumor activity. Int. J. Oncol., 15, 1155-1162, 1999). Briefly,

10^7 IGROV1 cells were injected in the peritoneal cavity of nude mice, and the compounds were administered i. v. at three doses on days 4 and 14 after tumor implantation. The anti-tumour activity was evaluated as described above for A549 lung carcinoma.

Results

Table 3 – In-vivo activities on C38, A549 and IGROV1 tumour models

Compounds	***Dose***	***T/C***
C38		
Example 7	25 mg/kg	12 %[a]
Example 8	50 mg/kg	15 %[a]
Example 11	25 mg/kg	41 %[a]
A549		
Example 7	12.5 mg/kg	218 %[b]
Example 8	25 mg/kg	340 %[b]
IGROV1		
Example 7	12.5 mg/kg	148 %[b]

[a] : tumor growth   [b] : survival

Compounds 7 and 8 are highly active against the three models used : they inhibit the growth of C38 colon adenocarcinoma by 85-88%, and markedly increase the survival of mice bearing two human solid tumours.

In conclusion, we have demonstrated that representative compounds of the instant application possess potent cytotoxicity on many cell lines and that they can be used in the treatment of tumors like leukaemia, carcinoma, adenocarcinoma, lung and ovarian carcinoma.

EXAMPLE 30 : Pharmaceutical composition : injectable solution

Compound of Example 11 .... 10 mg

Distilled water for injectable preparations .... 25 ml